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Baculovirus infection influences host protein expression in two established insect cell lines

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ABSTRACT

We identified host proteins that changed in response to host cell susceptibility to baculovirus infection. We used three baculovirus–host cell systems utilizing two cell lines derived from pupal ovaries, Hz-AM1 (from *Helicoverpa zea*) and Hv-AM1 (from *Heliothis virescens*). Hv-AM1 cells are permissive to *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and semi-permissive to *H. zea* single nucleopolyhedrovirus (HzSNPV). Hz-AM1 cells are non-permissive to AcMNPV. We challenged each cell line with baculovirus infection and after 24 h determined protein identities by MALDI TOF/TOF mass spectrometry. For Hv-AM1 cells, 21 proteins were identified, and for Hz-AM1 cells, 19 proteins were newly identified (with 8 others having been previously identified). In the permissive relationship, 18 of the proteins changed in expression by 70% or more in AcMNPV infected Hv-AM1 cells as compared with non-infected controls; 12 were significantly decreased and 6 cellular proteins were significantly increased. We also identified 3 virus-specific proteins. In the semi-permissive infections, eight proteins decreased by 2-fold or more. Non-permissive interactions did not lead to substantial changes in host cell protein expression. We hypothesize that some of these proteins act in determining host cell specificity for baculoviruses.

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1. Introduction

Concern about the long-term sustainability of agriculture is driving interest in biological control programs to manage pest insect populations. Baculoviruses are among the most important potential microbial control agents and are sometimes called biopesticides. Baculoviruses comprise a large family of arthropod-specific viruses, the Baculoviridae, composed of four genera, the Alphabaculovirus (lepidopteran-specific *Nucleopolyhedrovirus* (*NPV*)), Betabaculovirus (lepidopteran-specific *Granulovirus*), Gammabaculovirus (hymenopteran-specific NPV) and Deltabaculovirus (dipteran-specific NPV) (Jehle et al., 2006). Baculovirus diseases have been recorded in over 500 insect species (Miller, 1997). All baculoviruses are obligate parasites of arthropod hosts, although the host ranges, in terms of numbers of host species permissive to productive infections, vary among baculovirus/host systems.

The most deeply investigated baculovirus is the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) with a wide larval host range of 39 lepidopteran species (Guo et al., 2005). Most baculoviruses have much narrower host ranges. For example, the *Spodoptera exigua* MNPV (SeMNPV) is a pathogen solely of *S. exigua* (Cheng and Lynn, 2009). The narrow host range of many baculoviruses is one of the important limitations on the potentials for broader use of baculoviruses as biological control agents. Because of these limitations, it is important to understand the cellular and molecular mechanisms operable in determining baculoviruses host ranges with a vision of learning how to manipulate them.

Host ranges are ultimately determined by the interactions between baculoviruses and their potential hosts. The gypsy moth cell line Ld652Y, for example, is non-permissive to AcMNPV infection. The non-permissive reaction is due, at least in part, to shutting down total cellular protein synthesis and, separately, to inducing apoptosis (Du and Thiem, 1997). The Ld652Y line is permissive to *Lymantria dispar* MNPV (LdMNPV) infection, another baculovirus that carries the gene *host range factor 1* (*hrf-1*; Thiem et al., 1996). *hrf-1* specifies a 25.7 kDa protein that somehow inhibits the global shutdown of protein synthesis in infected cells, thereby allowing viral replication (Du and Thiem, 1997). Recombinant AcMNPV expressing the LdMNPV *hrf-1* efficiently replicated in Ld652Y. The single viral gene *hrf-1* acted to convert the non-permissive cell line Ld652Y into a permissive line. Later work similarly showed that Ld652Y cells are non-permissive to *Hyphantria cunea* NPV (HycuNPV), *Bombyx mori* NPV and *Se*MNPV. For all three baculoviruses, Ld652Y cells became permissive to recombinants bearing *hrf-1* (Ishikawa et al., 2004). The authors concluded that *hrf-1* is essential for nucleopolyhedrovirus

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replication in this cell line. *hrf-1* appears to be a genuine host range-influencing gene because it also expanded the AcMNPV host range to gypsy moth larvae (Chen et al., 1998).

Other baculovirus genes influence viral development in host cells. For example, expression of the AcMNPV host cell-specific factor 1 gene (*hcf-1*) is a required element for viral growth in TN368 cells derived from the looper *Trichoplusia ni*, but not required in Sf21 cells (Lu and Miller, 1996). The product encoded by *hcf-1* is a self-associating protein whose specific role in baculovirus replication remains unclear (Wilson et al., 2005). The key point is that some baculovirus genes facilitate viral development in some specific cell lines, and larval hosts, but not others. These baculoviral genes act to determine baculovirus host ranges.

Host cells also influence host range of baculoviruses. The *Thysanoplusia orichalcea* MNPV (ThorMNPV), for example, productively infects two lepidopteran species, *Pseudoplusia includens* and *T. ni* via *per os* treatment. Wang et al. (2008) found that the baculovirus infected hemocytes, tracheae and Malpighian tubules, but not fat bodies of non-permissive *S. frugiperda*, *S. exigua* or *Helicoverpa zea* larvae. Using the established Sf21 cell line, they also recorded very slow cell infection rates. By comparing larval death following *per os* and intrahemocoelic injection, they found *per os* treatments did not kill the larvae, while injection treatments did. The authors inferred that the primary midgut infections generated insufficient baculoviruses. Wang et al. (2008) concluded that these three factors, slow cell infection rates, inefficient primary infections at the midgut level and failure to replicate in the larval fat bodies limited the ThorMNPV host range. While they did not address the cellular mechanisms acting in these factors, their work makes the point that events registered at the whole-larva level act in host range determination.

Host gene products can also limit baculovirus host range. The silkworm, *B. mori*, was regarded as being non-permissive to AcMNPV infections. Guo et al. (2005) studied AcMNPV infectivity in larvae of 31 genetic strains of *B. mori*, finding 14 permissive strains. Increased viral titers indicated AcMNPV replicated in the permissive strains. The authors also conducted genetic cross experiments, from which they inferred *B. mori* has one dominant anti-AcMNPV gene, or perhaps a set of genes, that blocks AcMNPV infection in the non-permissive strains.

Both baculovirus and host cell genes and their proteins act in determining host specificity. Several baculovirus genes that confer advantage to the virus in infecting hosts and in neutralizing host defense reactions have been discovered (Thiem, 2009). In this paper, we examined the protein expression of several host cells in response to different baculoviruses. We hypothesized that infection by different baculoviruses influenced the expression of host cell proteins based on host cell susceptibility.

2. Materials and methods

2.1. Insect cell lines and baculoviruses

We used two cells lines, both derived from pupal ovaries, BCIRL-Hz-AM1 cells (Hz-AM1), derived from *H. zea* and BCIRL-Hv-AM1 cells (Hv-AM1) from *Heliothis virescens* (McIntosh and Ignoffo, 1983). Both cell lines were authenticated by DNA Fingerprinting-PCR (McIntosh et al., 1996). The cells were maintained at 28 °C in Ex-Cell 420 medium (SAFC, St. Louis, MO) containing 10% heat-treated fetal bovine serum (Summit Biotechnology, Ft. Collins, CO) with penicillin (100 U/ml) and streptomycin (100 g/ml; HyClone, Logan, UT). AcMNPV-HPP, cloned from a wild-type AcMNPV (McIntosh et al., 1992), was produced in Hv-AM1 cells and HzSNPV/Br (Goodman et al., 2001) was produced in Hz-AM1 cells.

2.2. Cell inoculation

We conducted experiments with three baculovirus–host cell systems, a permissive (high occlusion body and high budded virus production), a semi-permissive (low to moderate occlusion body and budded virus production), and a non-permissive system (no occlusion body and negligible to no budded virus production) (Bishop et al., 1995). Hv-AM1 cells were permissive to AcMNPV infection and semi-permissive to HzSNPV infection (McIntosh et al., 1985). Hz-AM1 cells were non-permissive to AcMNPV infection. Each cell line was seeded into a T-75 cm^2 flask with 1.35×10^7 cells and allowed to attach overnight. The medium was exchanged with 1 ml of fresh medium alone (control) or with inoculum of multiplicity of infection 1.0 for 2 h (rocker platform, setting = 3; Bellco Biotechnology, Vineland, NJ). Without removing the inoculum, both infected and mock-infected flasks were replenished with 15 ml fresh media, incubated for 24 h and then processed for protein analysis.

2.3. 2D gel electrophoresis and MS/MS analysis

Detailed procedures for gel electrophoresis, trypsin digestion and MS/MS analysis have previously been described (Stanley et al., 2008). The following alterations were made to the published procedure. After performing 2D gel electrophoresis, gel images were captured on a VersaDoc Imaging System (Model 4000, Bio-Rad). Spot matching, spot quantitation, and significant differences between treatments (Student's *t*-test, $P < 0.05$) were determined using Delta2D ver. 3.6 software (DECODON GmbH BioTechnikum, Greifswald, Germany, http://www.decodon.com/). For the permissive and semi-permissive treatments, protein spots that had densities significantly different between treatments were removed using a 1.5 mm spot picker (The Gel Company, San Francisco, CA). For the non-permissive treatments, since only moderate changes were noted, proteins either that showed statistical variations between treatments, or had high densities with gel-to-gel variations of less than 10%, were isolated. All protein spots were stored at −80 °C and removed at a later date for trypsin digestion.

Once digested, proteins were analyzed in the positive ion mode by reflector MALDI TOF/TOF (4700 MALDI TOF-TOF, Applied Biosystems, Foster City, CA) and MS/MS of the 10 most intense ions acquired (with $S/N > 20$ per digest) using automated data acquisition. Spectra were batch analyzed using Applied Biosystems GPS Explorer software (vers. 3.0). Database searches were performed with Matrix Science's search engine (http://www.matrixscience.com/) against the NCBInr Metazoa and Virus protein databases. Subsequently, mass data for proteins with low Molecular Weight Search (MOWSE) scores (Pappin et al., 1993) were used to interrogate a *Heliothis* database (Shelby and Popham, 2009) or the NCBI EST_other database. *In silico* protein identification criteria included protein score, expect level, percent sequence coverage, mass error, and sequence contiguity. Ion scores were generated for individual matched peptides (only highest scoring shown), as well as total ion scores. These data, combined with observed MW and pI values of the cell line proteins, were used to establish protein identities. Database searches for one HvAM1 protein did not yield significant matches using Mascot; therefore manual *de novo* sequence analysis was performed. Sequences generated from this analysis were used to interrogate NCBI-BLASTp using the PAM30 matrix and searching within "Insecta". *E*-values and frequency of matches to a specific protein were the primary criteria for these determinations.

Three independent biological replicates (1 gel/replicate) were conducted for each of the control and baculovirus–host cell treatments. Biological significance of changes in spot densities was set at 2-fold or more.

3. Results

Representative gels of cell lysates of permissive, semi-permissive and non-permissive relationships 24 h post-treatment are displayed in Fig. 1. For the permissive relationship, Fig. 1A shows proteins from control Hv-AM1 cell lysates and Fig. 1B shows proteins in cell lysates after exposure to AcMNPV. These two gels appear similar to the gels from the semi-permissive Hv-AM1/HzSNPV relationship (Fig. 1E and F). Gels prepared from the non-permissive Hz-AM1/AcMNPV relationship are shown in Fig. 1C and D.

With respect to the permissive and semi-permissive systems, many of the proteins seen in the lysates of control cells were not present or perhaps only present at low levels in lysates of baculovirus infected cells (Fig. 1B and F).

In the permissive relationship AcMNPV infection led to substantial changes in expression 18 Hv-AM1 cell proteins. The proteins were organized in terms of cellular functions, including signal transduction, protein action, cell protection and lipid metabolism (Table 1). Of these, expression of 12 proteins was reduced by approximately 70% or more, including protein spots 9, 10, 22, 4, 1, 2, 23, 24, 5, 20, 21 and 7. Large increases (5- to 8-fold) in expression of six of the cell line proteins were recorded. Additionally, we identified two proteins that were only slightly modified in their expression levels in response to virus infection.

Although the gels of lysates prepared from semi-permissive infections of Hv-AM1 cells appear superficially similar to the gels prepared from permissive infections of the same cell line, the semi-permissive infections led to entirely different changes in protein expression. Of the 14 proteins we selected for identification from these gels eight were substantially decreased (by 2-fold or more) (Table 1).

The non-permissive infections of Hz-AM1 cells did not lead to a substantial reduction in the total numbers of protein spots on 2D gels (Fig. 1C and D). Four cell proteins, numbers 57, 66, 55, and 60 slightly increased in expression by approximately 50–90% (Table 2). Small reductions (<2-fold) were recorded in expression of 10 other proteins.

Identifications of proteins from Hv-AM1 cells by *in silico* databank matches of mass spectrometric data are presented in Tables 3 and 4 for the *Heliothis* EST and the NCBI Virus databases, respectively. Virus-specific proteins were clearly distinguished from host cellular proteins in that they had significant MOWSE and total ion scores in the Virus database search and non-significant scores in the Metazoa database search. One Hv-AM1 protein,

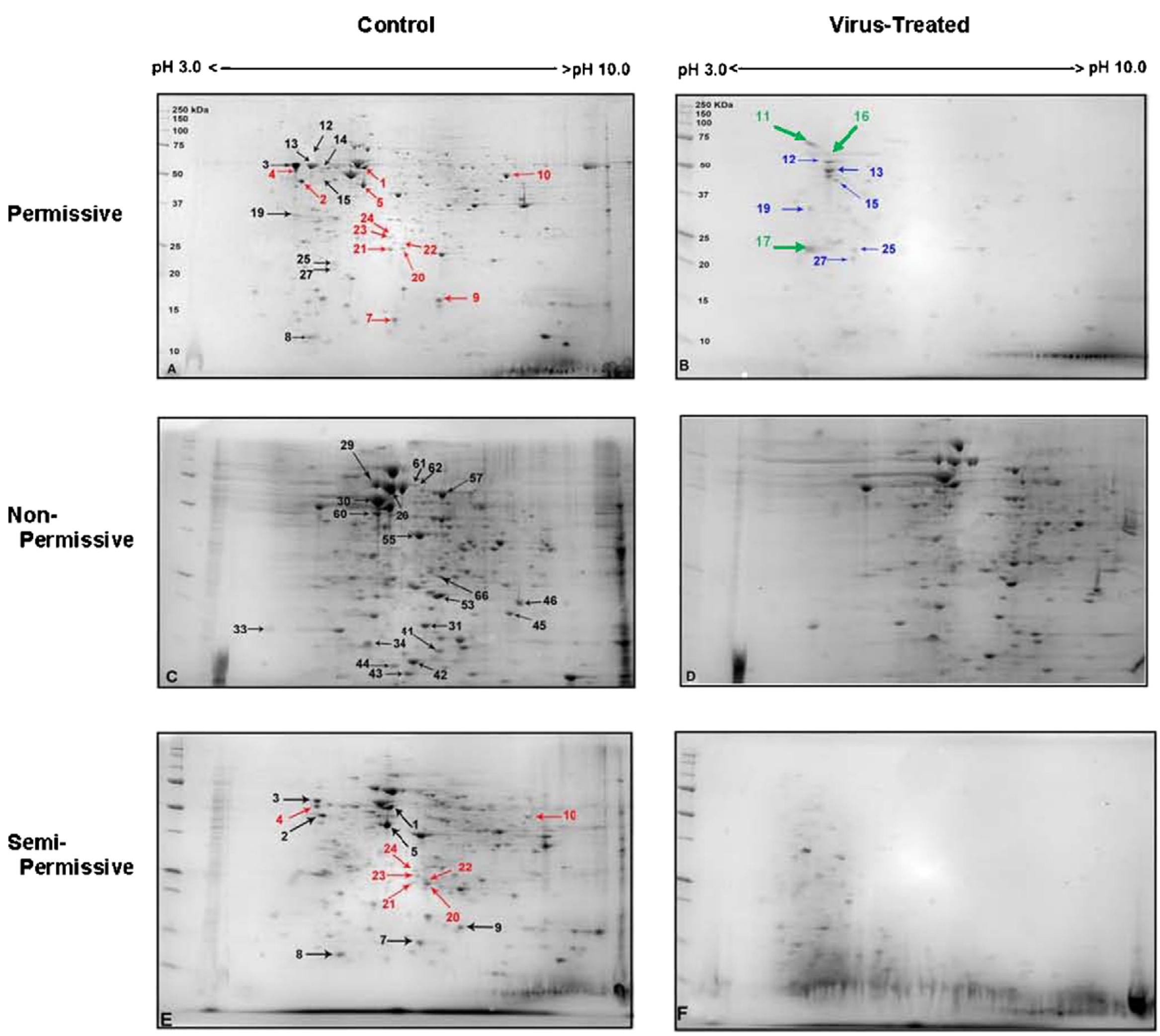

Fig. 1. Representative gels showing expression at 24 h post-infection in cell lysates of (A) control Hv-AM1 cells; (B) AcMNPV infected Hv-AM1 cells (permissive infection); (C) control Hz-AM1 cells; (D) AcMNPV infected Hz-AM1 cells (non-permissive infection); (E) control Hv-AM1 cells; and (F) Hv-AM1 infected with HzSNPV (semi-permissive infection). Numbers indicate sample spots that were extracted from gels and subjected to MS/MS analysis. In panels A and E, red numbers indicate proteins that decreased more than 2-fold following baculovirus infection. In panel B, blue numbers indicate proteins that increased more than 5-fold following baculovirus infection. Additionally, in Panel B, green numbers indicate proteins that were only found in AcMNPV-infected cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Relative quantity and function of proteins identified by MS/MS from Hv-AM1 cells permissive to AcMNPV challenge and semi-permissive to HzSNPV challenge.

Category	AcMNPV/HvAM1		HzSNPV/HvAM1			
	Spot no.	% of control[a]	Spot no.	% of control[1]	Putative protein name	Function
Energetics/metabolism	8	104.1	8	79.4	Thioredoxin-like protein	Electron carrier; important for cell redox homeostasis
	9	16.5	9	68.9	Abnormal wing-disk protein	Catalyzes conversion of NDP to NTP
	10	29.2	10	16.3	Acetyl-CoA hydrolase/transferase	Catalyzes the formation of acetate and/or succinyl-CoA
	22	19.1	22	25.9	Triosephosphate isomerase	Important in several metabolic pathways; essential for efficient energy production
Signal transduction	3	71.9	3	50.9	Calreticulin	Calcium binding protein that prevents misfolded proteins from being exported from the endoplasmic reticulum to the Golgi apparatus
	4	0.3	4	42.4	Calreticulin	Calcium binding protein
	12	651.9			Calreticulin	Calcium binding protein
	13	865.8			Calreticulin	Calcium binding protein
	14	71.9			Calreticulin	Calcium binding protein
Transcription	2	33.1	2	62.8	DNA supercoiling factor	Role in transcriptional activation via alteration of chromatin structure
	15	500.0			DNA supercoiling factor	Role in transcriptional activation via alteration of chromatin structure
	19	679.5			DNA supercoiling factor	Role in transcriptional activation via alteration of chromatin structure
Protein action	1	6.0	1	81.7	Disulfide-isomerase like protein ERP57	Interacts with two lectin chaperones and thereby promotes the oxidative folding of newly synthesized glycoproteins
	23	6.0	23	21.2	hsp21.4 protein	Folding and assembly of proteins
	24	8.3	24	5.8	Hypothetical protein AaeL_AAEL004438 (GrpE)	Folding and assembly of proteins
	25	575.5			Heat shock protein 70 kDa	Folding and assembly of proteins
	27	712.5			Heat shock protein 70 kDa	Folding and assembly of proteins
Cell function	5	11.4	5	101.7	Cytoplasmic actin A3a2	Involved in numerous functions, including: cytoskeleton structure, cell mobility, chromosome movement and muscle contraction.
Cell protection	20	11.2	20	48.6	Glutathione-S-transferase	Detoxification/metabolism
	21	10.9	21	17.9	Thioredoxin peroxidase	Protection from peroxides; possible role in signal transduction (via H_2O_2 or superoxide ions)
Lipid metabolism	7	13.6	7	90.0	Fatty acid binding protein 3	Fatty acid (unsaturated) binding

[a] Relative percent change in the protein spot density between those cells mock-infected and those challenged with virus, excluding background.

number 24, was not identified via this means and therefore was subjected to *de novo* sequence analysis. The protein identity from this analysis was the hypothetical protein AaeL_AAEL004438 (*Aedes aegypti*), gi|157106034 (*E*-value = 0.16), a GrpE protein which functions as a nucleotide exchange factor for DnaK-type Hsp70 s. Other search results confirmed this identity.

Similarly protein identifications were made from the Hz-AM1 cell line using Mascot in conjunction with the NCBI metazoa database (Table 5) and the *H. virescens* EST database (Table 6). Eight Hz-AM1 cell line proteins detected in this study were previously identified by Stanley et al. (2008) and are presented in Table 7.

4. Discussion

The data reported in this paper supports our hypothesis that baculovirus infection modulates protein expressions that correlate to different susceptibilities of host cell lines in response to infection by different baculoviruses. Baculovirus infections led to measurable differences in expression of a range of proteins in two cell lines at 24 h post-infection. We considered the possibility that the baculovirus influence on host cell protein expression is limited to one or another category of baculovirus/host cell interaction. Contrarily, we found that baculovirus challenge influenced host cell protein expression during permissive, semi-permissive and non-permissive infections. We infer from these findings that baculovirus/host cell interactions lead to specific changes in host cell protein expression based on susceptibility.

Although permissive, semi-permissive and non-permissive infections resulted in alterations in host cell protein expression, our results with permissive and semi-permissive infections of the same host cell line reveal six proteins that were very highly expressed in the permissive, but not the semi-permissive, infections (spots 12, 13, 15, 19, 25, and 27). We hypothesize that the expression of these proteins facilitates host cell permissiveness.

In the permissive Hv-AM1/AcMNPV interaction, we recorded substantial changes in expression of proteins involved in signal transduction, protein actions and cell protection. Four proteins involved in calcium binding, two for calreticulin (spots 12 and 13) and two for a DNA supercoiling factor (spots 15 and 19), increased in expression by 5- to 8.6-fold. DNA supercoiling factors generate unrestrained negative DNA supercoils in a Ca^{2+}-dependent manner and are known to regulate transcription (Ohta et al., 1995, Ogasawara et al., 2007). In mammals, calreticulin is a multifunctional ER protein that operates to maintain ER homeostasis (Ni and Lee, 2007). Calreticulin serves as a chaperone and assists in the folding of new glycoproteins. It also acts in intracellular signaling by binding calcium within ER stores. In some cancers, calreticulin

Table 2
Relative quantity and function of proteins identified by MS/MS from Hz-AM1 cells non-permissive to AcMNPV challenge.

Category	Spot no.	% of control[a]	Putative protein name	Function
Protein action	26	93.6	Heat shock cognate 70 kDa[b]	Folding and assembly of proteins
	29	83.7	Heat shock cognate 70 kDa[b]	Folding and assembly of proteins
	30	80.6	Heat shock protein 60 kDa[b]	Folding and assembly of proteins (can assist in virus production)
	34	112.7	Ubiquitin-like protein SMT3[b]	Modulation of protein activities
	57	169.9	70 kDa heat shock protein/90 kDa organizing protein-like protein	Folding and assembly of proteins
	61	74.3	Heat shock cognate 70 kDa	Folding and assembly of proteins
	62	78.5	Heat shock cognate 70 kDa	Folding and assembly of proteins
Cell protection	44	78.3	Mitochondrial thioredoxin 2	Mitochondrially localized antioxidant and antiapoptotic protein
	45	82.9	Mn superoxide dismutase[b]	Protects from reactive oxygen species
	46	92.2	Glutathione-*S*-transferase-like protein[b]	Detoxification/metabolism
	53	117.0	Thioredoxin peroxidase	Protects from peroxides; possible role in signal transduction (via H_2O_2 or superoxide ions)
Lipid metabolism	42	68.2	Fatty acid binding protein 3	Fatty acid (unsaturated) binding
Cell function	31	58.1	Actin-depolymerizing factor 1	Cell movement and cell division
	41	113.8	Deoxyuridine 5′-triphosphate nucleotidohydrolase	Hydrolyzes dUTP to dUMP and pyrophosphate; important for DNA replication and the limiting of intracellular pools of dUTP
	43	34.2	Profilin	An actin-binding protein involved in the dynamic turnover and restructuring of the actin cytoskeleton
	66	185.3	Bmsqd-1	A nuclear RNA-binding protein: involved in transcription, nuclear pre-mRNA processing, cytoplasmic mRNA translation and turnover
Energetics/metabolism	55	152.1	Arginine kinase	Catalyzes the transfer of phosphate from ATP to arginine
	60	145.5	26S protease regulatory subunit 6B	Responsible for the regulated degradation of intracellular proteins
Signal transduction	33	87.9	Calmodulin[b]	A calcium binding protein that is important in many regulatory processes

[a] Relative percent change in the protein spot density between those cells mock-infected and those challenged with virus, excluding background.
[b] Stanley et al. (2008).

can appear on the cell surface and serves as a tag for cell removal. Viral infection is one of the stressors that operate to perturb ER function and calreticulin may operate in maintaining ER functions during viral replication. Because this protein serves a variety of cell functions it is not surprising to see several calreticulin protiens. We note, too, that two other calreticulins, spots 3 and 4, were down-regulated during the permissive viral infection. We hypothesize that the up- and down-regulation of specific calreticulin proteins is a sign of the precision of baculovirus influence on host cell protein expression.

Two heat shock proteins, a 70 kDa heat shock protein (spot 27) and a heat shock protein cognate protein (spot 25), were up-regulated by 5- to 7-fold at 24 h following permissive infection. These proteins serve as chaperones, assisting in protein folding. They also stabilize and refold stressed proteins and they act in the degradation of irreversibly damaged proteins. Whereas calreticulin and related proteins act in the ER lumen, heat shock proteins generally act in the cytosol (Routsias and Tzioufas, 2006). We also note two enzymes (spots 10 and 22) involved in energy metabolism were substantially down-regulated. Protein spot 10 is an acetyl-CoA hydrolase/transferase and has also been found in the shrimp *Artemia franciscana* and in *Drosophila*. In these arthropods the enzyme influences cell structure and organization by acting in cross-linking microtubules (Oulton et al., 2003). Many enzymes in glycolysis are organized by microtubules and microtubule cross-linking may influence energy metabolism. Spot 22 is a triosephosphate isomerase, which operates in glycolysis to catalyze the reversible introversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. Aside from its importance in energy metabolism, this is a model enzyme in the study of molecular evolution (Tyshenko and Walker, 1997).

Permissive baculovirus/host cell interactions are, by definition, interactions that lead to efficient production of new viral progeny. While this process will culminate in the death of the host cell, premature cell death, that is, death before development of viral progeny, could fatally interrupt the viral life cycle. We speculate that baculovirus success in permissive infections is favored by up-regulation of selected proteins involved in cell signaling, transcription, and protein action which are likely vital to baculovirus production. However a subsequent down-regulation of proteins involved in cell protection, cell function and lipid metabolism which may function to maintain the life of the host cell likely occurs to move the cell resources toward baculovirus production and not host cell maintenance and survival.

The semi-permissive HzSNPV/Hv-AM1 interactions also resulted in reduced number of protein spots from host cells. However, calreticulin and other proteins just discussed were not expressed in the HzSNPV infected cells at 24 h post-infection. The absence of these proteins during the infection cycle may be limiting factors in the semi-permissive HzSNPV/Hv-AM1 interactions and possibly semi-permissive baculovirus/host cell interactions generally.

The non-permissive AcMNPV/Hz-AM1 cell interactions did not result in the general reduction of total protein spots seen in permissive and semi-permissive interactions. There was a slight reduction in expression of most of the 19 proteins we identified,

Table 3
Proteins from Hv-AM1 cells identified by *in silico* analysis using Mascot and the *Heliothis* EST database.

Spot no.	Putative protein with accession no. (species)	EST designation	Mass[a]	pI[a]	MOWSE score[b]	Total ion score[c]	No. peptides[d]	% Protein Coverage[e]	*E*-value[f]	Peptide Sequence (with highest score)[g]
1	Disulfide-isomerase like protein ERp57 (*Bombyx mori*) AB210112.1	EY120054.1	55,087	5.3	512	367	19	68	4.1e−044	IKGEVSQEYNGPR (89)
2	DNA supercoiling factor (*Bombyx mori*) NM_001043872.1	EY120867.1	37,894	4.5	500	385	16	53	6.5e−043	QFDHDAFLGEDQAK (94)
3	Calreticulin (*Galleria mellonella*) AB063250.1	EY119298.1	46,024	4.5	435	342	17	38	2.1e−036	FYNNPEEDKGLQTSEDAR (110)
4	Calreticulin (*Galleria mellonella*) AB063250.1	EY119294.1	46,024	4.5	254	189	9	39	2.6e−018	FYNNPEEDKGLQTSEDAR (102)
5	Cytoplasmic actin A3a2 (*Helicoverpa zea*) AF286061.1	EY120805.1	41,870	5.3	639	438	25	52	5.7e−61	SYELPDGQVITIGNER (102)
7	Fatty acid-binding protein 3 (*Helicoverpa armigera*) EU325560.1	600575002F1	14,848	6.4	396	220	17	58	1.6e−032	AIGVGLITR (59)
8	Thioredoxin-like protein (*Manduca sexta*) AF117573.1	EY119822.1	11,753	4.8	133	81	7	74	3.3e−006	IEEFSGANVDKLR (81)
9	Abnormal wing-disk protein (*Bombyx mori*) NM_001099814.1	600526321F1	17,313	6.8	488	356	13	77	1e−041	QMLGATNPADSLPGTIR (88)
10	GJ14323 Acetyl-CoA hydrolase/transferase (*Drosophila virilis*) XM_002058380.1	GR961557.1	51,900	7.3	190	115	10	32	6.5e−012	AHVNYVVTEQGIADLFGK (86)
12	Calreticulin (*Bombyx mori*) AB090887.1	EY119299.1	45,769	4.5	418	281	16	39	1e−034	FYNNPEEDKGLQTSEDAR (119)
13	Calreticulin (*Bombyx mori*) AB090887.1	EY119298.1	45,769	4.5	637	407	25	47	1.3e−056	FYNNPEEDKGLQTSEDAR (100)
14	Calreticulin (*Bombyx mori*) AB090887.1	EY119298.1	45,769	4.5	398	273	26	53	1e−032	FYNNPEEDKGLQTSEDAR (86)
15	DNA supercoiling factor (*Bombyx mori*) NM_001043872.1	EY120867.1	37,894	4.5	353	285	14	47	3.3e−028	TFDQLPPEESKR (74)
19	DNA supercoiling factor (*Bombyx mori*) NM_001043872.1	EY120867.1	37,894	4.5	217	167	9	36	1.3e−014	HLVFEADTDADKLTK (88)
20	Glutathione S-transferase GSTX01 (*Helicoverpa armigera*) EF591059.1	600584741F1	23,748	5.8	223	107	17	78	3.3e−015	AAQVDYEPIEEVKESR (104)
21	Thioredoxin peroxidase (*Bombyx mori*) NM_001046999.1	EY122473.1	25,284	8.3	290	227	10	55	6.5e−022	TISQDYNVLLDAGFALR (118)
22	Triosephosphate isomerase (*Bombyx mori*) AY736358.2	EY120750.1	26,392	5.7	409	271	14	66	2.1e−042	NWLSSNASPDVAASVIR (106)
23	Hsp21.4 protein (*Bombyx mori*) NM_001043520.1	EY118320.1	21,404	5.8	265	71	17	66	2.1e−019	DGVLTVEAPLPQLAITDR (44)
25	Heat shock protein 70 (*Helicoverpa armigera*) FJ432703.1	EY118112.1	71,618	5.2	185	127	9	37	2.1e−011	LSKEEIER (51)
27	Heat shock protein 70 (*Helicoverpa armigera*) FJ432703.1	EY118115.1	71,618	5.2	182	141	9	35	1.0e−013	ITITNDKGR (66)

[a] Determined using the ExPASy Compute pI/MW tool, http://ca.expasy.org/tools/pi_tool.html.
[b] MOWSE protein scores > 91 are significant ($P < 0.05$).
[c] Ion scores > 54 are significant ($P < 0.05$).
[d] This is the number of peptide sequences identified by Mascot that contributed to the MOWSE score.
[e] The percent of the protein sequence which is accounted for by the matching sequences.
[f] *E*-values generated by Mascot.
[g] Peptide sequence with the highest ion score shown in brackets.

Table 4
Proteins from Hv-AM1 cells identified by *in silico* analysis using Mascot and the NCBI Virus database.

Spot no.	Putative protein with accession no. (species)	Mass[a]	pI[a]	MOWSE score[b]	Total ion score[c]	No. peptides[d]	% Protein coverage[e]	*E*-value[f]	Peptide Sequence (with highest score)[g]
11	Telokin-like protein-20 (AcMNPV) C_001623.1	19,752	4.5	336	248	9	28.0	1.5e−28	RGVGAHIIK (63)
16	Chitinase (*Plutella xylostella* MNPV) NC_008349.1	61,527	4.8	616	305	39	58	1.5e−56	TIPGSFESLQR (79)
17	Telokin-like protein-20 (AcMNPV) NC_001623.1	19,752	4.5	246	221	6	19.0	1.5e−19	RGVGAHIIK (57)

[a] Determined using the ExPASy Compute pI/MW tool, http://ca.expasy.org/tools/pi_tool.html.
[b] MOWSE protein scores > 70 are significant ($P < 0.05$).
[c] Ion scores > 40 are significant ($P < 0.05$).
[d] This is the number of peptide sequences identified by Mascot that contributed to the MOWSE score.
[e] The percent of the protein sequence which is accounted for by the matching sequences.
[f] *E*-values generated by Mascot.
[g] Peptide sequence with the highest ion score shown in brackets.

but we did not record very large increases in any of the Hz-AM1 proteins at 24 h pi. We infer these proteins are not involved in the non-permissiveness of the AcMNPV/Hz-AM1 cell interaction. Presumably the non-permissiveness is due to other aspects of this baculovirus/host cell interaction.

Baculovirus infection is known to alter the protein production in host cells. AcMNPV was shown to cause a global shutoff of host protein synthesis at approximately 18 h post-infection and virus polypeptides to appear as early as 3 h post-infection (Carstens et al., 1999). Host RNA levels were found to be reduced from approximately 12–18 h post-infection and included host proteins actin, histone, and heat shock 70 (Ooi and Miller, 1988). Using differential display, Nobiron et al. (2003) confirmed a global down-regulation of mRNA levels in AcMNPV infected Sf9 cells and found several mRNAs that were up-regulated during early times of infection including the Hsc70 gene.

Eight proteins detected in this study (Table 7) were previously shown to change in response to prostaglandins in Hz-AM1 cells (Stanley et al., 2008). In particular, three heat shock proteins, believed to have a role in protein folding and assembly, were each found to decrease in expression following 24 h exposure to prostaglandin E_1. Three proteins we identified, a fatty acid binding protein, actin A3, and heat shock protein 60, were also identified in highly resistant *B. mori* strains to BmNPV infection (Bao et al., 2009). Further qPCR quantitation of actin A3 highlighted a much lower protein expression in susceptible larvae than in resistant larvae (Bao et al., 2009) which mirrors the lower protein expression we saw in our permissive system.

A number of host proteins that respond to baculovirus infection have been identified in recent years. Baculovirus infection increased the expression of Hemolin, a lepidopteran-specific immunoglobulin protein, in the Chinese oak silkmoth *Antheraea pernyi* (Hirai et al., 2004). Gene fragments encoding a protein named REPAT were identified in *S. exigua* larvae that increased in expression in response to baculovirus infection (Herrero et al., 2007). Several proteins with activity against BmNPV have been found to be secreted into the digestive juice of *B. mori* larvae, including a serine protease (BmSP-2), a lipase (BmLipase-1), and the red fluorescent proteins of mulberry fed larvae *via* a light-dependent mechanism (Yao et al., 2006).

Identification of host genes responding to viral challenge has recently been explored in an EST library and several microarray studies. Using an EST library generated from hemocytes isolated from baculovirus infected *H. virescens* (Shelby and Popham, 2009), several transcripts were identified including an ortholog to the BmNPV-induced *B. mori* Sop2 protein (suppressor of profilin 2; Xu et al., 2005), a *B. mori* secreted protein that is acidic and rich in cysteine, and is induced by BmNPV or densonucleosis infection of

Table 5
Proteins from Hz-AM1 cells identified by *in silico* analysis using Mascot and the NCBI Metazoa database.

Spot no.	Putative protein with accession no. (species)	Mass	pI	MOWSE score[a]	Total ion score[b]	No. peptides[c]	% Protein coverage[d]	*E*-value[e]	Peptide sequence (with highest score)[f]
31	Actin-depolymerizing factor-1 (*Bombyx mori*) NM_001099808.1	17,227	6.2	461	338	15	82	1.4e−40	YIQATDLSEASQEAVEEK (117)
42	Fatty acid-binding protein 3 (*Helicoverpa armigera*) EU325560.1	14,848	6.4	627	485	16	81	3.6e−57	FKPGEEFDEERADGAK (100)
43	Profilin (*Bombyx mori*) NM_001043643.1	13,875	5.9	91	76	3	22	0.0015	AAIAGHDGNVWAK (76)
44	Mitochondrial thioredoxin 2 (*Bombyx mori*) NM_001046818.1	17,039	9.0	200	174	4	40	1.8e−14	LVGLQDTDKLR (90)
53	Thioredoxin peroxidase (*Helicoverpa armigera*) EU131680.1	22,122	5.9	215	136	15	82	5.7e−16	GLFIIDDKQNLR (102)
55	Arginine kinase (*Epicephala* sp.) FJ235407.1	27,604	6.1	206	112	23	82	4.5e−15	NWGDVETLGNLDPAG EFVVSTR (49)
57	Hsp70/Hsp90 organizing protein-like protein (*Glossina morsitans*) DQ377065.1	56,438	7.9	248	239	13	27	2.8e−19	ALELDPSNAEALEGYR (112)
60	26S protease regulatory subunit 6B (*Bombyx mori*) NM_001046873.1	47,167	5.1	602	449	42	73	1.1e−54	EAVELPLTHVELYR (104)
61	Heat shock protein 70 (*Spodoptera exigua*) FJ609645.1	75,041	5.8	559	450	43	58	2.3e−50	NAVITVPAYFNDSQR (116)
62	Heat shock protein 70 (*Spodoptera exigua*) FJ609645.1	75,041	5.8	647	488	57	66	3.6e−59	NAVITVPAYFNDSQR (121)

[a] MOWSE protein scores > 75 are significant ($P < 0.05$).
[b] Ion scores > 45 are significant ($P < 0.05$).
[c] This is the number of peptide sequences identified by Mascot that contributed to the MOWSE score.
[d] The percent of the protein sequence which is accounted for by the matching sequences.
[e] *E*-values generated by Mascot.
[f] Peptide sequence with the highest ion score shown in brackets.

Table 6
Proteins from Hz-AM1 cells identified by *in silico* using Mascot and a selected EST database[a,b].

Spot no.	Putative protein with accession no. (species)	EST designation	Mass[c]	pI[c]	MOWSE score	Total ion score	No. peptides[d]	% Protein coverage[e]	*E*-value[f]	Peptide sequence (with highest score)[g]
41	Deoxyuridine 5′-triphosphate nucleotidohydrolase (*Aedes aegypti*) XM_001658230.1	ES582861.1[a]	16,072	5.9	292	238	8	56	3.2e−22	NFIDVGAGVIDEDYR (106)
43	Profilin (*Bombyx mori*) NM_001043643.1	600494224F[b]	13,875	5.9	164	134	6	26	2.6e−009	AAIAGHDGNVWAK (76)
44	Mitochondrial Thioredoxin 2 (*Bombyx mori*) NM_001046818.1	600591063F[b]	16,879	9.0	243	174	9	61	3.3e−017	LVGLQDTDKLR (90)

[a] Searched NCBI EST _others database; MOWSE protein scores > 90 are significant ($P < 0.05$); ion scores > 58 are significant ($P < 0.05$).
[b] Searched *Heliothis* database; MOWSE protein scores > 91 are significant ($P < 0.05$); ion scores > 54 are significant ($P < 0.05$).
[c] Determined using the ExPASy Compute pI/MW tool, http://ca.expasy.org/tools/pi_tool.html.
[d] The number of peptide sequences identified by Mascot that contributed to the MOWSE score.
[e] The percent of the protein sequence which is accounted for by the matching sequences.
[f] *E*-values generated by Mascot.
[g] Peptide sequence with the highest ion score shown in brackets.

Table 7
Hz-AM1 cellular proteins previously identified *in silico*[a].

Spot no.	Putative protein with accession number (species)
26	HSC 70 (*Trichoplusia ni*) U23504.1
29	Heat shock cognate 70 protein (*Spodoptera frugiperda*) AY161271.1
30	Heat shock protein 60 (*Culicoides variipennis*) U87959.1
33	Calmodulin (*Patinopecten* sp.) P02595
34	Ubiquitin-like protein SMT3 (*Bombyx mori*) DQ311411.1
45	Mn superoxide dismutase (*Bombyx mori*) NM_001043834.1
46	Glutathione-*S*-transferase-like protein (*Galleria mellonella*) AF336288.1
66	Bmsqd-1 (*Bombyx mori*) D38013.1

[a] Stanley et al. (2008).

B. mori midgut (Bao et al., 2009), and orthologs of the BmNPV-inducible paralytic peptide binding protein of *B. mori* (Hu et al., 2006). Following *per os* infection of the lightbrown apple moth, *E. postvittana*, with EppoNPV, microarray and further qPCR identified 21 host genes that were strongly up-regulated and 31 that were strongly down-regulated (Gatehouse et al., 2009). Six of the up-regulated genes and three of the down-regulated genes were involved in transcription (Gatehouse et al., 2009). In a *B. mori* cell line, the transcriptional profile of host genes during the early phase of BmNPV infection found 35 genes significantly up-regulated and 17 genes significantly down-regulated. Further qPCR quantitation confirmed that the expression of 13 genes significantly increased and 7 genes (including HSP90 and Hsp20.1) significantly decreased after BmNPV infection (Sagisaka et al., 2010).

Baculoviruses have been used in integrated pest management programs, mostly outside of the United States (Moscardi, 1999). The idea of improving the effectiveness of baculovirus bioinsecticides is a broad mandate involving detailed understanding of baculovirus/host interactions (Thiem, 1997) and practical engineering of deployable products (Szewczyk et al., 2006; van Beek and Davis, 2007). Our identification of specific host cell proteins possibly involved in permissiveness of baculovirus/host interactions is essentially an hypothesis that guides continued research in each of these proteins and their expression over the course of infection.

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